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AD____________

Award Number: DAMD17-01-1-0575

TITLE: Adenoviral Vectors Incorporating Multiple Targeting and Efficacy Strategies to Eliminate Breast Cancer Metastases

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REPORT DATE: July 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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Nov 12, 2002

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2002	Final (1 Jul 01 - 30 Jun 02)

4. TITLE AND SUBTITLE
Adenoviral Vectors Incorporating Multiple Targeting and Efficacy Strategies to Eliminate Breast Cancer Metastases

5. FUNDING NUMBERS
DAMD17-01-1-0575

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, Jul 02). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

Gene transfer of p53 induces cell death in most cancer cells, and replication-defective adenoviral vectors expressing p53 are being evaluated in clinical trials. However, low transduction efficiency limits the efficacy of replication-defective vector systems for cancer therapy. The use of replication-competent vectors for gene delivery may have several advantages, holding the potential to multiply and spread the therapeutic agent after infection of only a few cells. However, expression of a transgene may adversely affect viral replication. We have constructed a replicating adenoviral vector (Adp53rc) that expresses high levels of p53 at a late time point in the viral life cycle and also contains a deletion of the adenoviral death protein (ADP). Adp53rc infected cancer cells demonstrated high levels of p53 expression in parallel with the late expression pattern of the adenoviral fiber protein. p53 expression late in the viral life cycle did not impair effective virus propagation. Survival of breast cancer cell lines was decreased after infection with Adp53rc, compared to an identical p53-negative control virus. p53 expression also improved virus release and spread. Interestingly, p53 was more cytotoxic than the ADP in cancer cells but less cytotoxic than the ADP in normal cells. In conclusion, late expression of p53 from a replicating virus improves tumor cell killing and viral spread without impairing viral replication. In addition, in combination with a deletion of the ADP, specificity of tumor cell killing is improved.

14. SUBJECT TERMS
Gene therapy, Breast Cancer, Adenovirus, p53

15. NUMBER OF PAGES 23

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT Unlimited
Unclassified	Unclassified	Unclassified	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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INTRODUCTION

Adenoviral vectors mediate gene transfer at a high efficacy compared to other vector systems, and they are frequently used as vectors for cancer gene therapy. The results of numerous clinical trials have shown that the safety profile of therapeutic adenoviruses is favorable, especially when compared to other forms of cancer treatment. However, the anti-tumor activity of most adenoviral-mediated gene therapy approaches has been disappointingly low (Sterman *et al.*, 1998; Herman *et al.*, 1999; Stewart *et al.*, 1999; Lamont *et al.*, 2000; Nemunaitis *et al.*, 2000; Weill *et al.*, 2000; DeWeese *et al.*, 2001; Nemunaitis *et al.*, 2001; Schuler *et al.*, 2001; Sung *et al.*, 2001; Harvey *et al.*, 2002). The overal aims of this proposal were therefore to construct a replicating adenoviral vector that was able to effectivley kill tumor cells, and also efficently spread from tumor cell to tumor cell. The specfic objectives were as follows: 1) Achieve efficacious tumor cell killing by the replication of a modified adenoviral vector with an E1b-19kD gene deletion and late expression of p53; 2) Achieve highly selective tumor cell targeting by controlled expression of a modified E1a gene. During the one-year of funded support, we have focused on the first objective and constructed a replication-competent adenoviral vector that expresses high levels of p53 late in the viral life cycle. We have shown that transgene expression mimics the late expression kinetics of the adenoviral fiber protein, and that maximum levels of expression depend on viral DNA replication. Further, late expression of p53 improves the cytotoxic and lytic properties of a replication-competent vector without impairing viral replication. In addition, we have shown that p53 expression can preferentially substitute for the cell-lytic function of the adenoviral death protein in tumor cells compared to normal cells, leading to enhanced specificity of tumor cell killing. Our plans for the next phase of this research include making an E1b19kD deletion in the viral backbone, and introducing a modified E1a gene under the control of a breast specific promoter.

BODY

Construction of Adp53, a replicating adenoviral vector expressing p53 from a late reading frame.

Ad5 DNA was isolated and the adenoviral fiber cDNA was amplified by PCR (sense: TGCAGATGAAGCGCGCAAGAC and antisense: AACACAAACGATTCTTT-ATTCTTGG) and ligated into pCR3.1 (Invitrogen, Carlsbad, CA). The fiber cDNA was then excised between flanking EcoR1 restriction sites and subcloned into the first multiple cloning site of pIRES (Clonetec, Palo Alto, CA). RNA was extracted from A549 cells (wild-type p53 sequence), reverse transcribed and the p53 cDNA was amplified by PCR (sense: CGTAC<u>TCTAGA</u>TCACT-GCCATGGAG and antisense: GAATGTCAGTCTGAGTCAGG) and cloned into pcDNA3.1/V5/His-Topo (Invitrogen). The sense primer included a 5' Xba1 linker (underlined), and the cDNA was excised between the Xba1 and the Not1 site (present in the plasmid's multiple cloning site) and subcloned into the second multiple cloning site of pIRES (Clontec). The Fiber-IRES-p53 sequence, including a polyA signal present in the IRES plasmid, flanked by Hpa1 restriction sites, was then excised and cloned into the Hpa1 restriction site (within the fiber gene) of the pAB27 shuttle plasmid (Microbix, Toronto, Canada). This plasmid consists of the right-hand end of the adenoviral genome and contains a deletion of the E3 region and therefore does not encode the adenoviral death protein. Hpa1 cuts the backbone towards the 3' end of the fiber sequence, and the fiber reading frame was restored by the fiber-IRES-p53 fragment. The modified pAB27 plasmid was then co-transfected with pFG173 (Microbix,), a plasmid that contains the Ad5 genome with a deletion between 75.9 mu and 84.9 mu, into 293 cells to obtain infectious virus. Virus purification and titration were performed on 293 cells using standard methods (Graham and Prevec, 1995). An identical, non-p53-expressing control virus (Ad-co) was also constructed by the co-transfection of unmodified pAB27 and pFG173.

Other control viruses include Ad309 (Ad5 *dl*309) (gift from Dr. T. Schenk, Princeton University, NJ) which is similar to wild-type virus but contains a deletion from Ad5 of bp 30005-30750. This deletion abrogates the expression of the E3 14.7K, 14.5K and 10.4K proteins, but the E3 11.6K (ADP) is expressed at normal levels (Bett *et al.*, 1995), as is E3 12.5k, 6.7k and gp19k.

Adcmvp53 (Ad5.CMV-hp53; InvivoGen, San Diego, CA) is a first generation E1a- and E1b-deleted adenovirus, expressing p53 under the control of a CMV promoter.

Adp53rc, a replication-competent adenovirus, expresses high levels of p53 late in the viral life cycle, matching the pattern of adenoviral fiber expression.

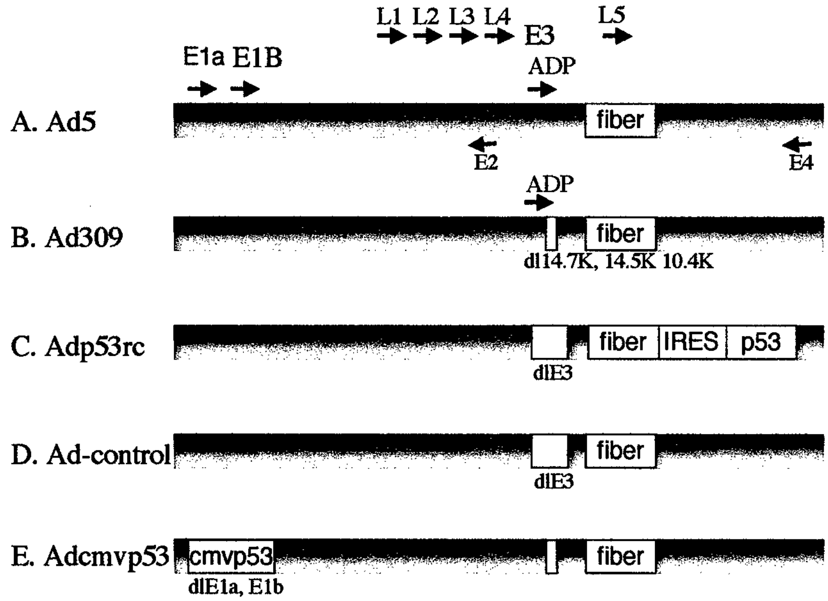

Figure 1. Schematic of the adenoviral genome of Ad5 and viruses used in this research. (A) Schematic of Ad5. The horizontal bar represents the genome and the arrows indicate relevant transcription units. (B) Ad309 is identical to Ad5 except for a small deletion/substitution within the E3 gene that abrogates the expression of the E3 14.7K, 14.5K and 10.4K proteins. The ADP remains expressed at normal levels. (C) Adp53rc has the p53 cDNA inserted into the adenoviral fiber transcriptional unit, using an internal ribosomal entry site. The virus also contains a complete E3-deletion and therefore does not express the ADP. (D) Ad-control (Ad-co) is identical to Adp53rc except for the IRES-p53 expression cassette. (E) Adcmvp53 is a first generation non-replicating E1a-deleted adenovirus that expresses p53 under the control of the CMV promoter.

Using replication-competent adenoviral vectors, it is desirable to express cytotoxic or cytolytic transgenes late in the viral life cycle, so that viral replication is not inhibited. The adenoviral fiber is one of the most abundant viral proteins which is expressed late, at the onset of viral DNA replication, and a similar pattern would be most suitable for transgene expression. To achieve p53 expression kinetics and levels similar to fiber, we constructed Adp53rc, an adenovirus that has the p53 cDNA inserted into the adenoviral fiber transcriptional unit, using an internal ribosomal entry site. After infection of p53-

negative H1299 cells with Adp53rc, the expression kinetics of p53, adenoviral fiber, E1a and E1b-55kD were examined by immunoblotting. The expression of the E1a protein, a product of an early viral gene, was detectable at 12 hr, reached a maximum at 18 to 24 hr, and was already greatly reduced at 36 hr post infection (Fig. 2).

In contrast, the p53 protein, tracking the pattern of fiber, was only weakly expressed at 18 hr and reached strong levels of expression after 36 hr. Maximum levels of p53 could be observed from 48 to 96 hr post infection. Cells infected with a p53-negative but otherwise identical control virus (Ad-co) did not show any p53 signal (data not shown). E1b-55kD peak expression level was observed at 48 hr post infection, but then rapidly declined and was almost undetectable at 96 hr.

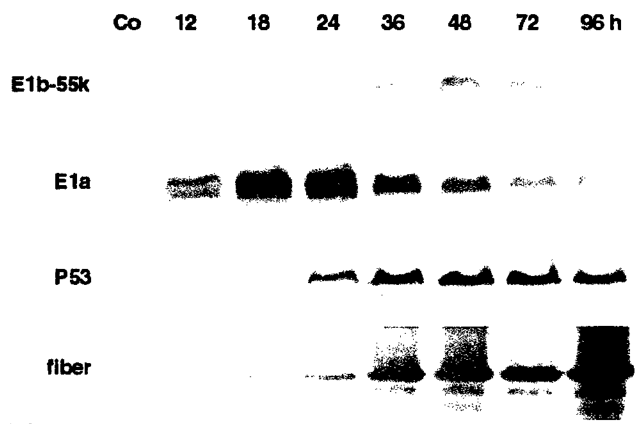

Figure 2. Time course of p53, adenoviral fiber, E1a and E1b-55kD expression from Adp53rc. H1299 cells were infected with Adp53rc at an MOI of 20 and harvested at the indicated time points. Cell lysates were subject to immunoblot analysis, with antibodies recognizing p53, E1a, fiber and E1b-55kD. Uninfected cells served as a control (co).

Expression levels of adenoviral late genes depend on viral DNA replication, and levels are reduced in the presence of cytosine arabinoside (Hawkins and Hermiston, 2001b), an inhibitor of DNA replication. If p53 transgene expression was to imitate the expression characteristics of fiber, it could be expected to also depend on viral DNA replication. Such expression kinetics could potentially be exploited to direct transgene expression towards tumor cells, if a targeting strategy that restricts viral replication to cancer cells was also added to the vector.

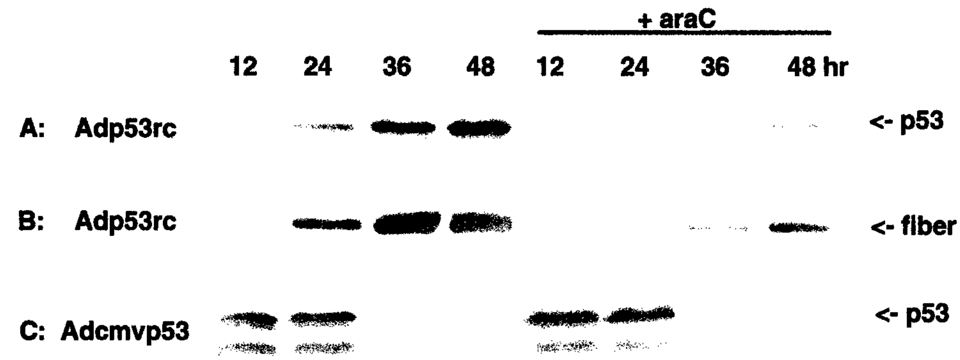

Figure 3. Late expression of p53 and adenoviral fiber is dependent on DNA replication. H1299 cells were infected with Adp53rc (lanes A and B) or with Adcmvp53 (lane C) with 20 PFU/cell in the presence or absence of cytosine arabinoside (araC), an inhibitor of DNA replication. Cells were harvested at the indicated time points, and lysates were subjected to immunoblot analysis with antibodies recognizing p53 or fiber.

To test if p53 expression from Adp53rc was dependent on viral DNA replication, we infected H1299 cells with Adp53rc in the presence or absence of cytosine arabinoside. Adcmvp53, an E1-deleted replication-deficient virus that expresses p53 under the control of a CMV promoter, was used as control. As expected, after infection with Adp53rc, p53 expression closely matched expression kinetics of fiber, and p53 expression levels were greatly reduced in the presence of cytosine arabinoside (Fig. 3), suggesting dependence of p53 protein levels on viral DNA replication. In contrast, p53 expression under the control of a CMV promoter was present at much earlier time points and was not affected by cytosine arabinoside.

p53 expressed from Adp53rc is located in the nucleus of infected cells

The E1b-55kD protein in a complex with E4orf6 is known to bind to and re-localize p53 into characteristic cytoplasmic clusters, leading to p53 degradation (Roth *et al.*, 1998). To analyze the intracellular localization of p53 following infection of H1299 with Adp53rc, the p53 protein was detected by immunofluorescence using a p53-recognising antibody. At 48 hr post infection, at the times of peak E1b-55kD protein expression, p53 was located predominantly in the nucleus, and only very small quantities could be detected in the cytoplasm (Fig. 4A). This expression pattern was very similar to control cells infected with an E1a- and E1b-deleted p53-expressing virus (Adcmvp53) (Fig. 4C). However, substantial nuclear export of p53 could be observed when wild-type virus (Ad309) was co-infected at twice the concentration of Adp53rc (Fig. 4B). This suggests that the transient peak levels of E1b-55kD protein expressed by Adp53rc

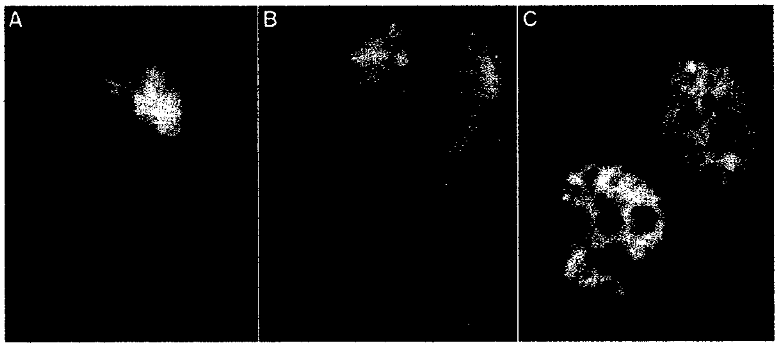

Figure 4. p53 expressed from Adp53rc is present predominantly in nuclear localization. H1299 cells were infected with Adp53rc (A), or Ad5cmvp53 at a MOI of 20 (C) or Adp53rc at a MOI of 20 plus Ad309 at a MOI of 40 (B). After 48 h (A,B,C) cells were harvested and p53 protein was visualized by immunofluorescent using an anti-p53 antibody. Cell nuclei were stained with DAPI. The experiment was performed twice, and representative cells are shown.

are insufficient to cause nuclear export and degradation of the highly expressed p53 protein.

Late p53 expression exhibits preferential cytotoxicity to breast cancer cells.

To be used as therapeutic agents against cancer, adenoviral vectors need to be efficacious in the elimination of cancer cells. Replication-competent adenoviruses display intrinsic oncolytic activity, which is however, insufficient for effective cancer therapy. To determine if late p53 expression could improve the oncolytic activity of such vectors, we infected H1428 and H2O2 p53-negative breast cancer cell lines and normal fibroblasts (IMR-90) with Adp53rc, Ad-co or Ad309 and measured cell survival at the indicated time points using a WST-1 assay (Fig. 5 & 6). Adp53rc was associated with increased tumor cell killing compared to Ad-co and Ad309 in the cancer cell lines (Fig 5). Differences at early time points were small, as would be expected when using a vector that depends on replication.

Although p53 gene transfer is cytotoxic to cancer cells, it has been shown to be relatively non-toxic to normal cells. In contrast, the E3-encoded adenoviral death protein, which is important for lysis of adenoviral-infected cells, may be cytotoxic to cancer and normal cells in a non-discriminate fashion. We hypothesized that late p53 expression from a virus that also contains a deletion of the death protein would have limited toxicity to normal cells and improve tumor-specific cell killing.

To investigate this hypothesis, we compared the cytotoxic effect of Adp53rc and the death-protein-expressing Ad309 in cancer cell lines and normal fibroblasts. Adp53rc was more cytotoxic than Ad309 in the p53-negative breast cancer cell lines (Fig. 5). This indicates that the cytotoxic function of late p53 expression not only substitutes for but is more effective than the cytotoxic function of ADP in cancer cells. In contrast, Adp53rc was significantly less cytotoxic than Ad309 in normal lung fibroblasts (IMR-90), comparing cell survival on days 4 to 10. This suggests that the cytotoxic effect of p53 is more tumor cell specific than the effect of the death protein.

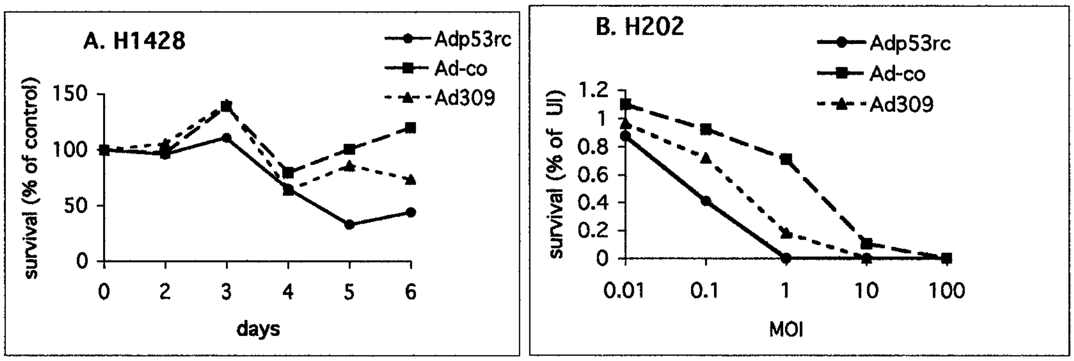

Figure 5. Breast cancer cell survival following infection with Adp53rc, Ad-co and Ad309.
H1428 breast cancer cells (A) were infected with 10 PFU/cell with Adp53rc, Ad-co, Ad309 or remained uninfected. Cell survival was evaluated by WST-1assay at the indicated time points. Survival of infected cells is expressed as a percentage of uninfected cells at the same time point. H2O2 (B) breast cancer cells were infected with the same viruses as in A, but at the MOI shown, and cell survival evaluated by WST-1 assay at the time when cytopathic effect was visible in the wild type (Ad309) control wells at an MOI of 1.

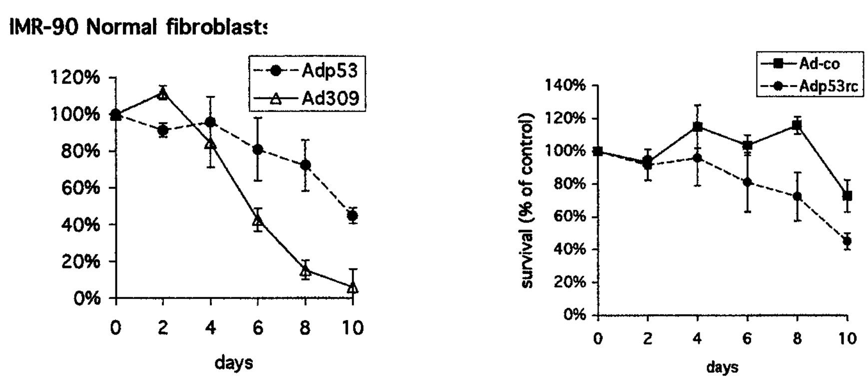

Figure 6. Survival of normal cells following infection with Adp53rc, Ad-Co and Ad309. IMR-90 cells were infected with 10 PFU/cell with Adp53rc, Ad309, Ad-Co or remained uninfected. Cell survival was evaluated by WST-1 assay at the indicated time points. Survival of infected cells is expressed as a percentage of uninfected cells at the same time point.

Late p53 expression enhances virus release in cancer cells but not in normal cells

Virus release and spread from cell to cell is critical for effective cancer therapy, as only a minority of tumor cells can be infected initially, even with direct intra-tumoral injection. The adenoviral death protein has an important function for cell lysis and viral release, and its over-expression has been shown to improve the oncolytic effect of replicating adenoviral vectors. However, as demonstrated in Figures 5 & 6, the cytotoxic effect of the death protein is less tumor cell specific than the effect of p53. Next, it was important to investigate if p53 expression could also substitute for the release and spread function of the death protein, and if this effect was specific to cancer cells.

To evaluate virus release, Calu-1 lung cancer cells and IMR-90 normal fibroblast cells were infected with Adp53rc, Ad309, or Ad-co and harvested on days 2 and 4. The amount of virus in the supernatant was then quantified by plaque assay on 293 cells. The results are shown in Figure 7. At 4 days after infection, supernatant of Calu-1 cells infected with Ad309 contained more than 6 times as much virus (1.7 x 10^6 PFU/ml) as supernatant of cells infected with Ad-co (2.7 x 10^5 PFU/ml). As these 2 viruses are almost identical except for the expression of the ADP, this difference can be attributed to the expression of the death protein and its known function of virus release. Supernatant of Adp53rc infected Calu-1 cells contained more than 5 x as much virus (1.4 x 10^6 PFU/ml) as

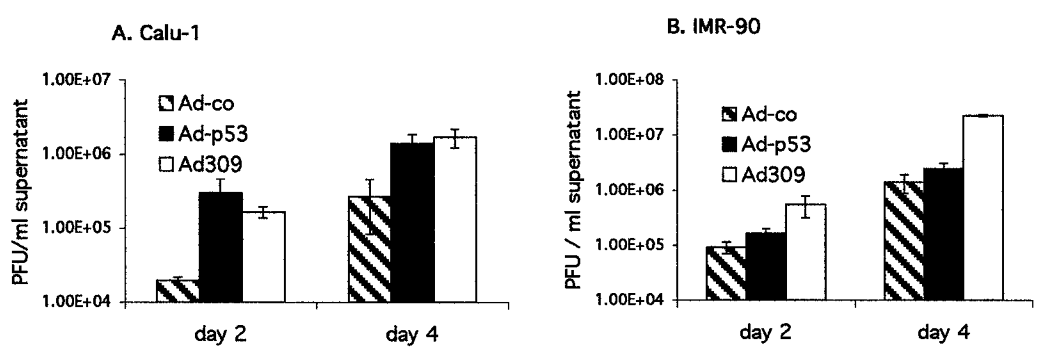

Figure 7. Virus release following infection with Adp53rc, Ad-co or Ad309. Calu-1 (A) and IMR-90 (B) cells were infected with Adp53rc (solid columns), Ad309 (open columns) or Ad-co (hatched columns) with 1 PFU/cell. Cells and supernatant were harvested separately on days 2 and 4 and the amount of virus in the supernatant was quantified by plaque assay on 293 cells. Each experiment was performed at least three times, and standard error bars are shown.

supernatant of Ad-co infected cell. These 2 viruses are identical except for the expression of p53, suggesting that p53 expression can substitute for the virus-release-function of the death protein in this cancer cell line. In IMR-90 cells, expression of the death protein by Ad309 was also associated with a large increase of virus release (2.23×10^7 PFU/ml), compared to Ad-co (1.41×10^6 PFU/ml). In contrast, virus release of Ad-co- and Adp53rc-infected cells (2.46×10^6 PFU/ml) was similar, suggesting that the effect of p53 expression on virus release is more cancer cell specific than the effect of the death protein.

p53 expression enhances virus spread in cancer cells more effectively than in normal cells.

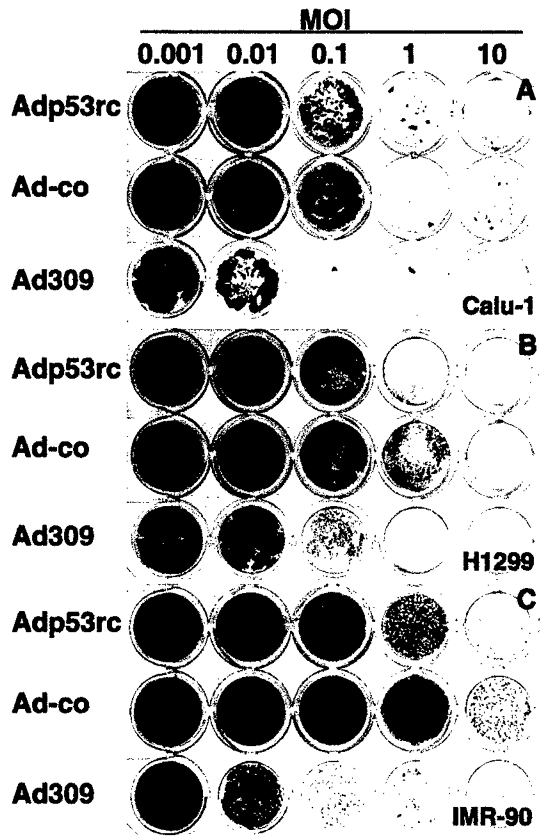

Figure 8. Virus spread following infection with Adp53rc, Ad-co or Ad309. Calu-1 cells (A), H1299 cells (B), and IMR-90 cells (C) were infected at log-fold dilutions starting with 10 PFU/cell. On day 8 (Calu-1, H1299) and day 12 (IMR-90) post infection, the remaining cell layer was fixed and stained with crystal violet. The experiment was performed twice, with similar results.

To assess if improved virus release would be associated with enhanced virus spread, we carried out a virus spread assay (Doronin *et al.*, 2000). The assay is based on the fact that at a low concentration (less than 1 PFU per cell) viruses need to go through at least one round of infection, replication, viral release and re-infection of surrounding cells in order to destroy a cell monolayer. H1299 cells, Calu-1 cells and IMR-90 cells were infected with log-fold dilutions of Adp53rc, Ad-co and Ad309. On day 8 (Calu-1, H1299) or day 12 (IMR-90) after infection, the remaining cell layer was stained with crystal violet.

p53 expression by Adp53rc and expression of the death protein by Ad309 was associated with improved viral spread in both cancer cells lines (Fig. 8). At a concentration of 0.1 PFU/cell, Adp53rc led to approximately 50%, and Ad309 to 100% elimination of the cell layer, while the cell layer infected with Ad-co remained intact.

However, in IMR-90 cells, p53 expression was associated with only minor enhancement of spread, whereas Ad309 led to a destruction of the monolayer at a 2-fold higher dilution than Ad-co-infected cells. This suggests that expression of p53 with a replicating virus preferentially leads to improved viral spread in cancer cells compared to normal cells. Expression of the death protein improves spread to a higher degree than p53 expression, but its effect is similar in both normal and cancer cells.

KEY RESEARCH ACCOMPLISMENTS

* Construction of a replicating adenoviral vector that expresses the p53 gene from a late reading frame in a death protein deleted viral backbone

* Demonstrating that p53 expression from this death protein deleted construct selectively improves breast tumor cell killing and viral release and spread.

REPORTABLE OUTCOMES

<u>p53 expression from a replication-competent adenovirus improves breast tumor cell killing. deletion of the death protein improves specificity.</u>
John G. Hay, Harald Sauthoff, Teona Pipiya, Sheila Heitner, Shu Chen and William N. Rom
Era of Hope, Department of Defense
Breast Cancer Research Program Meeting
Orange county convention center, Orlando, Florida
September 25-28, 2002

<u>Late Expression of p53 from a Replicating Adenovirus Improves Tumor Cell Killing and is More Tumor Cell Specific than Expression of the Adenoviral Death Protein</u>
Harald Sauthoff, Teona Pipiya, Sheila Heitner, Shu Chen, Robert Norman, William N. Rom and John G. Hay.
Revised manuscript submitted to Human Gene Therapy.

<u>Teona Pipiya,</u> Research Associate, was funded from this proposal.

CONCLUSIONS

Replication-competent viral vectors for cancer gene therapy have the potential to multiply a therapeutic agent up to a thousand fold in each infected cell. Repeated cycles of infection, cell lysis and virus spread to neighboring cells may eventually lead to elimination of all cells within a tumor. Wild-type adenovirus is lytic to infected cells, and tumor-targeted replicating adenoviruses are under clinical investigation (Nemunaitis *et al.*, 2000; DeWeese *et al.*, 2001). However, the intrinsic oncolytic properties of wild-type adenovirus are insufficient to eliminate established tumors (Harrison *et al.*, 2001). In this research, we have explored the use of a transgene to enhance the limited viral spread and oncolytic effect of a replication-competent adenoviral vector.

We have demonstrated that insertion of a transgene into the adenoviral fiber transcription unit, with the use of an internal ribosomal entry site, results in an expression pattern that mimics that of adenoviral fiber. Fiber makes up a large percentage of all proteins produced by the host cell at late stages of viral infection, and high levels of late transgene expression can be achieved with this system. In contrast to current adenoviral vectors with early transgene expression under the control of an exogenous promoter, our vector has the advantage of late transgene expression without the space requirements for an exogenous promoter. Late transgene expression may be important if the transgene is potentially deleterious to effective virus production. Further, maximum transgene expression under the control of the major late promoter is dependent on viral DNA replication, and should therefore be limited in normal cells if a targeting strategy that restricts viral replication to cancer cells is also adopted.

In recent years several investigators have utilized replication-competent adenoviral vectors for transgene delivery. Suicide genes, such as the HSV-tk gene (Wildner *et al.*, 1999) or a HSV-tk-cytosine-deaminase fusion gene (Freytag *et al.*, 1998; Rogulski *et al.*, 2000; Lee *et al.*, 2001), have been expressed under the control of exogenous promoters or the adenoviral E3 promoter (Lambright *et al.*, 2001), leading to amplification and spread of the prodrug-converting enzyme. However, the activated prodrug may limit viral replication and spread, and this may affect the efficacy of this approach. Cytokines, such as TNF-α have also been expressed in a tumor-targeted fashion, resulting in an increased oncolytic effect of the vector (Kurihara *et al.*, 2000).

Acknowledging the importance of the timing of transgene expression, Hawkins *et al.* recently described a gene delivery system utilizing the endogenous adenoviral gene expression machinery. By replacing various E3 transcription units with a transgene, this group demonstrated transgene expression kinetics similar to the substituted gene (Hawkins and Hermiston, 2001a; Hawkins and Hermiston, 2001b; Hawkins *et al.*, 2001).

Although many different types of transgenes have been shown to have anti-tumor efficacy, p53 has been studied extensively and proven efficacious in a large variety of human cancer types (Liu *et al.*, 1994; Mujoo *et al.*, 1996; Nielsen *et al.*, 1997). In addition, p53 gene transfer has been shown to have very little effect on normal cells, such as bone marrow cells, lymphocytes and normal fibroblasts (Zhang *et al.*, 1995; Scardigli *et al.*, 1997; Liu and Gazitt, 2000; Kawabe *et al.*, 2001). However, p53 expression from a replicating adenovirus may be complicated by multiple interactions between virus biology and p53 function. p53 can induce cell cycle arrest and apoptosis in infected cells, and it has been suggested that p53 may therefore limit viral replication (Bischoff *et al.*, 1996). Although limitation of viral replication by p53 has recently been debated (Koch *et al.*, 2001), the multiple mechanisms by which adenoviral proteins oppose p53 function suggest a deleterious effect of p53 on the viral life cycle.

Our vector was designed to express p53 late in the viral life cycle to prevent any possible inhibition of viral replication by p53. Furthermore, separation of maximum E1a and E1b gene expression from maximum p53 expression, based on the early and late expression kinetics of the E1 proteins and fiber respectively, was intended to prevent inactivation of p53 by early viral proteins. We were able to demonstrate strong p53 expression in nuclear localization and uninhibited viral replication. Although some nuclear export of p53 was observed, expression levels of p53-inactivating genes at times of p53 expression were insufficient to cause significant p53 degradation, which was expressed at high levels in the nucleus. Furthermore, p53 expression was associated with improved tumor cell killing, induction of apoptosis, virus release and spread.

Surprisingly, we were unable to demonstrate p53-dependent transactivation of downstream genes such as p21 or bax. E1b-55kD and E1a are known to inhibit p53-dependent transactivation (Steegenga *et al.*, 1996; Somasundaram and El-Deiry, 1997; Martin and Berk, 1998), and it is possible that low levels of these proteins were sufficient to inhibit p53-dependent transactivation. Our preliminary data indicate that the introduction of an E1a mutation that inhibits p300 binding restores p53-dependent p21 transactivation (data not shown). However, transcriptional activation of p53-target genes is dispensable for p53-dependent apoptosis and cell killing (Caelles *et al.*, 1994; Haupt *et al.*, 1995) and, depending on the cell line, the level of apoptotic response induced by transactivation-inactive p53 mutants may be reduced (Chen *et al.*, 1996) or augmented (Kokontis *et al.*, 2001). It is therefore difficult to predict if a vector with transactivation-active p53 would be more efficacious than our vector.

E1b-55kD-deleted vectors have been shown to be impaired in their oncolytic activity (Hall *et al.*, 1998; Harada and Berk, 1999; Hay *et al.*, 1999), but it may be possible to restore p53 transactivation activity by a single amino acid substitution within E1b-55kD (Shen *et al.*, 2001) or by modifications of E1a (Somasundaram and El-Deiry, 1997) or the p53 transgene (Lin *et al.*, 1994; Koch *et al.*, 2001). Furthermore, the oncolytic activity of our vector could probably be improved by the introduction of an E1b-19kD deletion. We have previously demonstrated that such a deletion improves the apoptotic function and virus spread of replicating adenoviruses (Sauthoff *et al.*, 2000), and the expression of this gene may be responsible for the relatively low levels of apoptosis induced by Adp53rc.

The E3-encoded adenoviral death protein is important for efficient lysis of the infected cells and release of adenovirus progeny. It is expressed at high levels late in the viral life cycle, and cell viability is prolonged when infected with an ADP-deleted mutant virus (Tollefson *et al.*, 1996). Utilizing the natural biology of the adenovirus, two groups have recently shown that the oncolytic potential of replicating adenoviruses can be enhanced by overexpression of this protein (Doronin *et al.*, 2000, Ramachandra, 2001 #435). In contrast to this approach, we deleted the ADP from our vector system to decrease toxicity to normal cells. We were able to show that late expression of p53 can substitute for the lytic function of ADP in cancer cells, leading to enhanced tumor cell killing and cell lysis. Although there was some increase in toxicity to normal cells associated with p53 expression, p53 is clearly more tumor cell selective than the ADP. Tumor-selective cell killing by late expression of p53 combined with an ADP deletion in a replicating adenovirus is a new concept, which could be synergistic with a targeting strategy that restricts viral replication to breast tumor cells.

John G. Hay, MD. Unpublished data.

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REPLY TO
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FOR THE COMMANDER:

Phyllis Rinehart

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